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REMARKS

Claims 1, 14-18, 80-83, and 87 have been amended. Claims 7, 13, 19, and 68 have been canceled by this amendment. Claims 88-122 are new. The amendments and new claims are supported throughout the application as filed, e.g., at page 2, lines 15-20; page 18, lines 18-20; page 18, lines 26-29; page 28, lines 27-31; and the original claims. Upon entry of this amendment, claims 1, 6, 7, 13-23, 53-68, 75-122 will be pending and under examination.

At the outset, Applicants thank Examiners Yu and Caputa for their time and thoughtful discussion during a telephonic interview conducted on July 8, 2003 with the undersigned. The substance of the interview is discussed in detail below.

Applicants acknowledge that the previous rejection of the claims under 35 U.S.C. § 103(a) is withdrawn.

Rejections under 35 U.S.C. § 112

Enablement

Claims 1, 6, 7, 13-23, 53-61, 63-68, 75, 76, and 84-87 were rejected under 35 U.S.C. § 112 as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

First, the Examiner maintained a previous rejection based on the argument that the specification does not teach the specific structures responsible for inhibiting endothelial cell migration. Second, the Examiner argued that the specification does not teach a method of treating a disorder using the active steps of the instant claims. Applicants' data, provided in a declaration filed with the response of October 1, 2002, were not included in the enablement analysis because the Examiner stated that it was unclear if the experiments in the declaration represented the administration of protein or DNA, and that the specification as originally filed did not describe the specific protein fragment. Third, the Examiner argued that therapy by administration of a protein is technically difficult. Fourth, the Examiner argued that the specification does not teach any *in vivo* treatment of any disease, and cited numerous references

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that describe the challenges of cancer therapy. This rejection is respectfully traversed. Each of the points made by the Examiner in this rejection is discussed below.

The present claims recite specific structures of TSP-2, which have been shown to work in the claimed methods

With regard to the Examiner's first point discussed above, the rejection has been met by narrowing claims 1 (from which other claims depend) and 87, to require that the fragment of TSP-2 comprise *at least 10 contiguous amino acids of either a procollagen domain of TSP-2, or a type I repeat of TSP-2*. Such fragments have been shown to work. As discussed in the interview, the present application provides sufficient guidance for a skilled artisan to practice the full scope of the claimed methods without undue experimentation. For example, the application provides a working example showing that TSP-2 inhibits tumor growth *in vivo* and shows that this activity correlates with the ability to inhibit endothelial cell migration *in vitro* and *in vivo*.

At least one fragment within the claims is described, which has the same ability to inhibit endothelial cell migration *in vitro*. In addition, in the first Declaration of Michael Detmar ("the first Declaration"), filed with the response of October 1, 2002, Applicants submitted data showing that an additional fragment that falls within the claims inhibits tumor growth and endothelial cell migration (this data is discussed in detail below). In addition, as was noted by Applicants in the response filed on October 1, 2002, the TSP-2 sequence is known, techniques for making fragments as recited in the claims are routine in the art, and the specification describes multiple assays which can be used to identify additional functional fragments. Therefore, the claims are adequately enabled.

The claims cover protein therapy with TSP-2 or fragments thereof, not gene therapy

With regard to the second point, the Examiner stated that the claims are not enabled because "the specification does not disclose any *in vivo* disorder treatment using the active step of the instant invention. The guidance and example in the specification is administering a

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nucleic acid molecule and there is no example or guidance about administering a protein." This basis for the rejection is respectfully traversed.

Contrary to the Examiner's assertion, there is ample support for administering a protein, as discussed in more detail below. While some of the experiments described in the application do include use of TSP-2 nucleic acids as tools to make TSP-2 protein, these examples demonstrate biological activities of TSP-2 protein and correlate with activities seen when the protein is administered. The present claims do not, on their face, cover gene therapy and are not intended to do so.

The *in vivo* assays in the application describe administration of TSP-2-transfected cells. See, for example, the xenograft assays described at page 33, line 17, to page 34, line 19. In these assays, nude mice were implanted with human epidermoid carcinoma cells of the A431 cell line, which had been transfected with TSP-2. The results of these assays show that TSP-2 expression inhibited growth of tumors by more than 90% after three weeks as compared to untransfected tumors. Similar results were obtained with human malignant melanoma cells of the MeWo cell line. These experiments reflect administration of protein *in vivo*. This is because TSP-2-expressing cells secrete TSP-2. The biological activities of TSP-2 are repeatedly described in contexts in which the protein is secreted. See, for example, page 32 lines 15-19, where the transfection and characterization of A431 cells is described as follows:

More than 10 stably transfected clones were obtained for each construct and confirmed efficient TSP-2 expression by Northern hybridization and by Western blotting of cell lysates and conditioned media. Importantly, TSP-2 was virtually absent in conditioned media obtained from confluent control A431 cultures transfected with vector only. (emphasis added)

TSP-2 transfectants release TSP-2 into the media. That cells secrete TSP-2 is apparent elsewhere in the specification, e.g., at page 16, lines 20-23, and page 32, lines 24-27. Furthermore, Applicants have demonstrated that one activity of TSP-2, the inhibition of endothelial cell migration, is caused by contacting endothelial cells with exogenously administered TSP-2 peptide fragments, and is not dependent on expression of a TSP-2 nucleic acid. See, e.g., the *in vitro* endothelial cell migration assay described at pages 39 and 40.

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Importantly, Applicants demonstrated that transfection of tumor cells with TSP-2 did not directly inhibit growth of the transfected tumor cells themselves. See page 32, lines 19-22, where it states that "no significant differences in cellular morphology and growth rates on plastic culture dishes, in soft agar colonization or in spontaneous and induced apoptosis rates were observed between control transfected and TSP-2 overexpressing A431 clones." In another assay, it was demonstrated that "anchorage-independent cell growth, as determined by the ability to form colonies in soft agar, showed no significant differences between TSP-2 transfected A431 clones and control transfected A431 clones" (page 41, lines 5-7).

Rather, the TSP-2 transfected tumor cells showed reduction in vascular vessel size and decreased vascular density *in vivo* (page 36, lines 3-12). That is, the TSP-2 transfected tumor cells were not themselves growth-inhibited, but rather, the growth and invasion of vascular vessels that feed the tumor was inhibited. Thus, the TSP-2 protein exerted effects *in trans*, on non-tumor cells such as endothelial cells of the vessel invading the tumor, as shown in these experiments. Thus, these assays demonstrate the activity of TSP-2 protein *in vivo*.

In addition to specific examples, general support for administration of a protein can be found, for example, at page 42, line 9, through page 46, line 9.

As requested by the Examiner during the interview, the data presented in the first Declaration of Michael Detmar, are discussed below. The data demonstrate efficacy of administration of protein *in vivo* using methods described in the application as filed.

Figure 1 of the first Declaration is a schematic diagram of the preparation of a recombinant protein fragment of TSP-2. As outlined in the text on the figure, a fragment of TSP-2 DNA, containing nucleotides 213-1883 of human TSP-2 cDNA was PCR amplified from a human cDNA library and cloned into an expression vector. This cDNA fragment encodes the procollagen domain and three type 1 repeat domains of TSP-2. The vector was transfected in mammalian 293 EBNA cells. Stably transfected clones were selected and propagated in medium containing puromycin. To purify the TSP-2 protein fragment encoded by nucleotides 213-1883 of TSP-2 (the TSP-2 N-terminal fragment, hereafter referred to as "TSP-2/NTF"), transfected 293 EBNA cells were cultured in serum-free medium. The medium was collected from the cells

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every two days until the cells began to detach. The medium was filtered and proteins were precipitated with ammonium sulfate and dialyzed. Next, the protein was purified by gelatin-sepharose and heparin-sepharose chromatography and dialyzed. Thus, the transfected cells in this experiment were merely used as a factory to make the TSP-2/NTF protein that was purified from the cell culture media. The protein was subsequently assayed *in vitro* and administered *in vivo*.

Figure 2 of the first Declaration is a graph showing the results of *in vitro* endothelial cell migration assays. These *in vitro* assays were performed essentially as described on page 39 of the specification with only minor technical changes. As shown in the graph, the greatest inhibition of migration was observed when the assay was conducted with cells that had been incubated in the presence of 1 μ g/ml TSP-2/NTF protein, although inhibition was also observed with cells treated with 0.5 and 2 μ g/ml TSP-2/NTF protein.

Figure 3 of the first Declaration presents results of *in vivo* treatment of tumor-challenged mice with phosphate buffered saline (PBS), endostatin, the purified TSP-2/NTF protein fragment (described above), and a fragment of the procollagen domain of TSP-2 (PC). Nude mice were injected intradermally with 2×10^6 A431 cells. Beginning 2 days after implantation of the A431 cells, mice were injected in the peritoneal cavity with 1 mg/kg purified TSP-2/NTF protein, PBS, endostatin, or PC. This was repeated daily for 18 days. The photo was taken 20 days after implantation of the tumor (i.e., after 18 days of treatment with the proteins). As is shown in the photo, treatment with TSP-2/NTF protein caused marked reduction of tumor growth relative to control (PBS). PC treatment also inhibited growth relative to control. Thus, systemic administration of a fragment of TSP-2 causes reduction of established tumors *in vivo*.

Figure 4 of the first Declaration is a graph showing the volume of A431 tumors over a period of 20 days in mice treated with PBS, endostatin, 1 mg/ml purified TSP-2/NTF protein, 0.01 mg/ml purified TSP-2/NTF protein, 1 mg/ml PC, and 0.1 mg/ml PC, as described for Figure 3. After 20 days, the volume of tumors in mice treated with 1 mg/ml TSP-2/NTF was approximately 700 mm³. The volume of tumors in control mice was approximately 1200 mm³ after the same amount of time. Treatment with TSP-2/NTF protein reduced the volume of

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tumors by approximately 40% over 20 days. PC treatment also reduced the volume relative to control. Figure 5 presents results of a similar experiment, in which 3 mg/kg of body weight of purified TSP-2/NTF protein was administered and compared to treatment with PBS. In this experiment, the treatment with TSP-2/NTF or PBS was initiated 4 days after implantation of the tumors. These are art-recognized models of tumor formation and treatment.

Thus, Applicants produced and expressed a TSP-2 protein fragment as claimed, using the methods and guidance provided in the specification and other methods routine to the skilled artisan. Applicants assayed the biological function of the protein fragment *in vitro* and confirmed its activity *in vivo*. As was noted in the first Declaration, the method for testing the protein fragment of TSP-2, the A431 tumor xenograft assay, was the same tumor xenograft assays described in the specification. Thus, this evidence shows that one of ordinary skill could readily perform the claimed method using the guidance provided in the specification, with no undue experimentation.

Protein therapy with TSP-2 or fragments thereof is enabled

The Examiner's third point in the enablement rejection was that the claimed methods are unpredictable because administration of protein is technically difficult e.g., due to proteolysis or challenges in administering protein to a target area. This basis for the rejection is respectfully traversed. Applicants' data clearly shows that TSP-2 protein and fragments thereof are effective to treat tumors. Quite simply, the claimed methods work *in vivo*.

U.S. Pat. 4,925,677 was cited as teaching that "albumin is degradable by proteolytic enzymes." Kastin, AJ (2001) was cited because it states that "peptides are degraded in different regions in rat cerebral microvessels." Frost, SJ (1993) was cited as teaching that "peptides are degraded by cell-surface peptidase activity on endothelial cells." The references cited by the Examiner provide no evidence that the claimed methods would not work. In fact, all of the references cited describe *in vitro* work only, while Applicants have shown, in multiple *in vivo* examples, that the claimed methods work *in vivo*. Further, it is unclear why one would correlate the susceptibility to proteolysis of other, completely unrelated proteins, to that of TSP-2.

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The claimed methods simply work to inhibit tumor growth

The Examiner's fourth point in the enablement rejection is that cancer therapy is unpredictable. This basis for the rejection is respectfully traversed. While it may be true that cancer therapy is *generally* unpredictable, Applicants are not claiming treating tumors with any and all methods generally. The present claims relate to a specific method, namely administering TSP-2 or specifically recited fragments thereof. Applicants have shown, in multiple examples, that the methods work to inhibit tumor growth *in vivo*. A skilled artisan, using the guidance provided in the specification, can readily perform the claimed methods. Nothing more is required.

The Examiner cited five references (discussed in more detail below) that discuss the difficulties of cancer therapy. Few of these references discuss anti-angiogenic therapy, much less therapy with TSP-2. The references disclose limitations in cancer therapies that do not apply to TSP-2 therapy at all.

Gura (1997) is concerned primarily with compounds identified by random screening, rather than therapeutics derived from naturally occurring proteins that regulate tumor growth. Jain (1994) examines the methods by which tumors resist penetration by drugs. Jain, however, does not teach that anti-angiogenic therapy is unpredictable. In fact, Jain teaches that such therapy may be a solution to the problem of drug penetration, thus supporting enablement of the claimed methods. See page 65, paragraph 6, where Jain states "alternatively, if a tumor's vascular system could be destroyed completely, no drug would have to extravasate or cope with the interstitium. The persistent, total lack of nourishment would be expected to starve and eventually kill tumor cells." Applicants have demonstrated this very effect *in vivo*. See, for example, page 35, lines 3-6, where necrosis of TSP-2-expressing tumors is described. Curti (1993) discusses chemotherapy and therapy with monoclonal antibodies, which is a completely different type of molecule than TSP-2. Curti does not discuss anti-angiogenic therapy such as that of the claimed methods. Curti does, however, state that "the key to understanding the unique properties of a tumor's physiology requires focus on the tumor vasculature and interstitial

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space" (at page 30, paragraph 3). The claimed methods target the vasculature of a tumor. Hartwell et al. (1997) discusses the utility of genetically tractable organisms in searching for cancer targets. Curti also discusses the problem of extrapolating data from non-mammalian organisms, such as yeast, to mammalian systems when designing anti-cancer therapeutics. These issues do not support non-enablement of the claimed methods, as the claimed methods have been shown to work *in vivo* on a mammalian model system. Bellone et al. (2000) is cited as teaching that DNA and protein therapeutics provide different, unpredictable results. Bellone et al. is concerned with immunological anti-cancer strategies, in which an animal's immune system is stimulated to react to tumor tissue. These strategies are also unrelated to the presently claimed methods of treatment, which relate to a different mechanism, namely angiogenesis.

The presently pending claims are directed to treatment of angiogenesis-dependent tumors. As discussed in the enclosed Second Declaration of Michael Detmar under 37 C.F.R. § 1.132, one of ordinary skill in the field of angiogenesis would understand that inhibition of one of the steps of angiogenesis would inhibit growth of any angiogenesis-dependent tumor. (An unsigned copy of the declaration is enclosed herewith for the Examiner's information. An executed copy will be submitted under separate cover shortly.) As such, one of ordinary skill would understand, based on the disclosed activities of TSP-2 and the data provided in the specification, that TSP-2 and the recited TSP-2 fragments can be used in methods of treating disorders characterized by tumors.

In summary, given the specific structures and functional limitations of the claims, the presence of numerous working examples (including multiple *in vivo* experiments), the high level of skill in the art, and the extensive guidance provided by the disclosure of the application, the present claims are enabled for their full scope.

Written Description

Claims 1, 6, 7, 13-23, 53-61, 63-68, 75, 76, and 84-87 are rejected under 35 U.S.C. § 112 as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed

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invention. The Examiner stated that "the Office maintains this rejection because the instant claims still lack written description of at least 90%-99% identical to SEQ ID NO:2 and also lack written description of a TSP-2 or its fragments capable of accomplishing the purpose stated in the preamble of the instant claims."

Applicants respectfully traverse the rejection as it may be applied to the presently pending claims. As discussed above, the claims have been amended to specify that at least a portion of the fragments comprise at least 10 contiguous amino acids of either a procollagen domain of TSP-2, or a type I repeat of TSP-2. Applicants have described multiple such fragments capable of treating a subject having a disorder characterized by angiogenesis-dependent tumor formation, as discussed above. As was discussed in the interview, TSP-2 or a fragment thereof having 90% identity to SEQ ID NO:2, wherein the fragment comprises at least 10 contiguous amino acids of either a procollagen domain of TSP-2, or a type I repeat of TSP-2, is adequately described because of the structure imposed by the high degree of identity and the requirement for specific domains. Applicants were clearly in possession of the full scope of the claims at the time of filing.

Moreover, Applicants direct the Examiner to Example 14 of the Revised Interim Written Description Guidelines Training Materials ("the Guidelines"). This Example describes a scenario in which a protein having at least 95% identity to a reference sequence, and a specific functional activity, is claimed. The Guidelines affirm the adequacy of the written description for this claim, even when only a single representative species is disclosed. The Guidelines state that the disclosure of a single protein species is sufficiently representative of the claimed genus because all members of the genus have high structural identity with the species, and because the Applicant provided an assay with which to identify variants having the recited high level of identity and the required functional property. The present claims are supported by an even greater amount of disclosed structural and functional information. The claims are directed to a genus having a very high degree of percent identity to a reference sequence, a required functional activity, and residues from specific regions of the protein. Furthermore, Applicants have disclosed multiple representative species, and the specification provides numerous assays for

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determining percent identity and functional activity. As the claims exceed the amount of written description support provided in Example 14, the claims are adequately described.

New Matter

The Examiner rejected claims 77-79 as containing subject matter which was not described in the such a way as to reasonably convey possession of the claimed invention. The Examiner asked that Applicants point to support for these claims. Claim 77 is directed to a method of treating a subject having an angiogenesis-dependent tumor, the method comprising administering to the subject a TSP-2 or a fragment thereof wherein the fragment comprises an amino acid sequence encoded by nucleotides 294-1367 of SEQ ID NO:1. This fragment is described at page 28, lines 27-28 of the specification. The nucleotide sequence of TSP-2 is SEQ ID NO:1, as is noted in Figure 1 and page 25, line 29. The fact that the tumors to be treated are angiogenesis-dependent is implicit, if not explicit, throughout the specification. See, for example, page 2, lines 15-20; and page 6, lines 10-11; page 26, lines 27-28.

Claim 78 is directed to a method of treating an angiogenesis-dependent tumor. The method includes administering to the subject a TSP-2 or a fragment thereof wherein the fragment comprises an amino acid sequence encoded by nucleotides 294-1883 of SEQ ID NO:1. This fragment is described at page 28, lines 29-31 of the specification.

Claim 79 is directed to a method of treating a subject having an angiogenesis-dependent tumor, the method comprising administering to the subject a TSP-2 or a fragment thereof wherein the fragment comprises an amino acid sequence encoded by nucleotides 1383-1883 of SEQ ID NO:1. This fragment is described at page 28, line 31, to page 29, line 1 of the specification.

In view of the foregoing, Applicants ask the rejection of the claims be withdrawn.

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The Commissioner is authorized to charge the any excess claim fees to deposit account 06-1050.

The Commissioner is authorized to charge the fees related to the enclosed Petition for Extension of Time in the amount of \$930 to deposit account 06-1050.

Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 30 July 2003

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